Green Synthesis and Characterization of Gold Nanoparticles Using Chitosan as the Reducing and Stabilizing Agent

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Abstract. The gold nanoparticles (AuNPs) were prepared with chitosan as stabilizer and reducing agent through a one-step green method. The size and morphology of AuNPs were characterized by UV-Vis spectra and transmission electron microscopy (TEM). The effects of reaction temperature, HAuCl₄ concentration, concentration and molecular weight of chitosan on the size and morphology of synthesized AuNPs were studied. According to the results, the spherical AuNPs with the average particle size around 10 nm could be prepared by 1 mL 1.0 mmol/L HAuCl₄ and 3 mL 0.01%(w/v) chitosan with molecular weight in the range from 48.75 to 3.99 kDa at 70°C. The reducing ability of chitosan for Au³⁺ was enhanced with the molecular weight decrease.

Introduction

Gold nanoparticles (AuNPs) have received considerable attention because of their unique optical, physical, chemical, electrical and catalytic properties[1,2]. Generally, AuNPs prepared by chemical reductants, such as citrate and boron hydride, from gold salts. However, these chemical agents used for the synthesis pose potential biological risks and make the synthesized AuNPs unsuitable for application in a biological environment. Therefore, there are a lot of researches aimed to develop green chemical reductants to achieve the controllable preparation of nanometer gold materials[3].

Chitosan, is the second most abundant natural biopolymer originated from chitin which is derived from the shells of shrimp and other crustaceans. Chitosan is a natural polysaccharide polymer rich in reactive amino and hydroxyl functional groups. It has been reported that chitosan can participate in both reduction and stabilization processes in the synthesis of colloidal AuNPs[4], avoiding the use of toxic reductants such as hydrazine and sodium borohydride.

In the paper, chitosan was used as both reducing and stabilizing agent to prepare AuNPs. Some factors, such as reaction temperature, HAuCl₄ concentration, concentration and molecular weight of chitosan on the size and morphology of as-synthesized AuNPs was investigated in detail.

Experimental Section

Materials

Chitosan was obtained from Shandong Aokang Biotechnology Co. and used to prepare chitosan with different molecular weight. The commercial neutral protease was obtained from Shanghai Solarbio Bioscience & Technology Co. Ltd. (China). All other chemical reagents were of analytical grade and used as received. Deionized water was used in all experiments.
The Preparation of CTS with Different Molecular Weight

Chitosan with the same degree of deacetylation but different molecular weight were obtained by enzymatic hydrolysis method. The properties of the chitosan samples are summarized in Table 1.

Table 1. Degradation condition and molecular parameters of chitosan hydrolysates.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DD (%)</th>
<th>([\eta]) (mL/g)</th>
<th>(M_v) ((\times 10^4))</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTS</td>
<td>94.9</td>
<td>553.0</td>
<td>48.76</td>
</tr>
<tr>
<td>DCs1</td>
<td>92.6</td>
<td>332.0</td>
<td>28.36</td>
</tr>
<tr>
<td>DCs2</td>
<td>94.3</td>
<td>72.3</td>
<td>4.60</td>
</tr>
<tr>
<td>DCs3</td>
<td>93.0</td>
<td>62.5</td>
<td>3.99</td>
</tr>
<tr>
<td>DCs4</td>
<td>93.5</td>
<td>5.4</td>
<td>0.24</td>
</tr>
</tbody>
</table>

The Preparation of AuNPs

All glassware were thoroughly cleaned with freshly prepared aqua regia solution (HNO₃:HCl=1:3, v/v) and rinsed extensively with deionized water before use. An amount of chitosan was completely dissolved in 1%(v/v) acetic acid. 3 mL of chitosan solution with constant stirring until the descried temperature was reached. Then, 1 mL of HAuCl₄ solution was added. The reaction was maintained under constant stirring for 2 h, unless described.

Characterization Methods and Instrumentation

Viscosity average molecular weight of chitosan was measured according to the method of literature [5]. The calculation was based on Mark- Houwink formula.

The DD of chitosan was determined by elemental analysis (EA) [6] and the DD can be calculated according Ref. UV-Vis spectra of gold nanoparticles in chitosan solutions were recorded using a spectrophotometer (TU-1810 UV/vis, Purkinje General Instrument Co., Ltd. Beijing, China).

Transmission electron microscopy (TEM) was carried out with H-7650 instrument (Hitachi High-Technologies Corporation).

Results and Discussion

The Influence of Reaction Temperature

The preparation of AuNPs is controlled by not only the reductant and stabilizer, but experimental parameters, such as temperature, reaction time, relative and absolute concentration of each reactant.

Fig 1. shows the UV-Vis spectra and TEM of AuNPs prepared with 3 mL 0.01%(w/v) CTS and 1.0 mmol/L HAuCl₄ for 2 h at different reaction temperature. The Au colloids color changed from colorless to wine red as the temperature increaed. A clear solution of wine red color is characteristic of spherical AuNPs of size around 20 nm obtained when the reaction temperature increased to 70°C, which indicated that there is no AuNPs synthesized at 60°C. It can be seen from the temperature-dependent UV-Vis spectra of AuNPs, the maximum absorption band appeared at 313 nm when the reaction temperature was 60°C. However, the maximum absorption band appeared at 520 nm when the reaction temperature increased to 70°C, which is the characteristic surface plasmon resonance of AuNPs. It was showed that CTS reduction efficiency could not make Au(III) completely turn into Au at 60°C. When the reaction temperature increased to 70°C, the Au colloids exhibits a single absorption band at 520 nm. It was generally considered the maximum absorption band appeared at 520 nm, showing that the spherical and quite dispersive AuNPs with even particle size distribution within the scope of 1~20 nm were obtained. With the
reaction temperature increased to 80°C, besides the intensity increase of the absorpition band, we noticed a slight red-shift from 520 to 533 nm, which could indicate an increase of particle size and a tendency towards aggregation.

Fig 1(B). and (C). shows TEM image of AuNPs prepared at 70°C and 80°C, respectively. AuNPs prepared at 70°C were for spherical particles with uniform shape, which was consistent with the results obtained from UV-Vis spectra. With the increase of reaction temperature, synthesized AuNPs become non-spherical particles and partial gathering appeared. The average particle size of AuNPs increased from 7.84±2.53 nm to 36.63±14.53 nm when temperature increased from 70°C to 80°C.

The Influence of HAuCl₄ Concentration

Fig 2. shows the effect of HAuCl₄ concentration on the synthesis of AuNPs. It was seen that the Au colloids was colorless as 0.5 mmol/L HAuCl₄ used. As HAuCl₄ concentration increased from 1.0 mmol/L to 2.0 mmol/L, the solution color changed from wine red to dark red. When the concentration increased to 3.0 mmol/L, the color of Au colloids changed as light yellow and some gold deposits was found in the reaction system. When HAuCl₄ was 0.5 mmol/L, the Au colloids was colorless and the maximum absorption band appeared at 313 nm, indicated that there is no AuNPs prepared. As HAuCl₄ concentration increased to 1.0 mmol/L, the Au colloids showed wine red and the maximum absorption band of Au colloids appeared at 525 nm, indicated the formation of individual AuNPs in solution[7]. Increased HAuCl₄ concentration to 2.0 mmol/L, the color of Au colloids changed to dark red and showed the maximum absorption band at 549 nm. Compared to 1.0 mmol/L HAuCl₄, besides the red-shift of the maximum absorption band, the half peak width of absorption spectrum also widened, indicating that the particle size distribution of AuNPs increased and a tendency towards aggregation. When the HAuCl₄ concentration increased to 3.0 mmol/L, the color of Au colloids changed as light yellow and some gold deposits was found in the reaction system. The maximum absorption peak appeared at 313 nm while the characteristics absorption peak of AuNPs disappeared, which may be due to CTS is not enough to stabilize a lot of gold nuclei, resulting in large accumulation and settlement of gold nuclei. The TEM image of AuNPs prepared with 2.0 mmol/L of HAuCl₄ showed spherical particles with larger size and partial aggregation, compared with the AuNPs synthesized by 1.0 mmol/L HAuCl₄.
The Influence of CTS Concentration

Fig 3(A). shows the UV-vis spectra of Au colloids prepared by different concentrations of CTS. There is no adsorption bands when 0.001% (w/v) CTS was used, indicated that the reduction efficiency of CTS is not enough for complete transformation of Au(III) into AuNPs at this concentration. As CTS solution concentration increased to 0.01%(w/v), the Au colloids showed the maximum absorption band at 525 nm. When CTS concentration increased to 0.1%(w/v), the maximum absorption peak intensity decreased and red shifted to 551 nm. At CTS concentration of 0.1%(w/v), more CTS was available to electrostatically attracted to and interwined around the negatively charged AuCl₄⁻ and AuNPs was encapsulated by CTS and more be embedded within CTS, contributing to a weaker AuNPs absorption peak at 551 nm. When the concentration of CTS increased from 0.1%(w/v) to 0.2%(w/v), the intensity of the maximum absorption band increased, and there was a slight blue shift of absorption band position. The results showed that the concentration change of CTS could change the particle size of the prepared AuNPs. Fig. 3 (B) and (C) are the TEM images of AuNPs prepared with 0.1%(w/v) and 0.15%(w/v) CTS, respectively. As could be clearly observed from them, synthesized AuNPs were mainly for spherical-like shape. The average particle size of AuNPs prepared with CTS of the concentration of 0.1%(w/v) and 0.15%(w/v) was 43.85±21.28 nm and 43.77±18.05 nm, respectively.

The Influence of CTS Molecular Weight

Fig 4 (A). is the UV-vis spectra of Au colloids prepared by 0.01%(w/v) of chitosan with different molecular weight. The Au colloids color changed from wine red to deep purple as the CTS molecular weight decreased from 48.76 to 0.24 kDa. When the chitosan molecular weight decreased from 48.75 kDa (CTS) to 3.99 kDa (DCs3), all the spectrum showed the maximum absorption bands at 525 nm, suggested that AuNPs were spherical. The intensity of absorption band increased very slightly with the CTS molecular weight decreased from 48.75 kDa (CTS) to 4.60 kDa (DCs2). When molecular weight reduced to 3.99 kDa (DCs3), the intensity of absorption peak increased significantly, indicated that the decrease of molecular weight enhanced the reducing capacity of CTS. When the molecular weight decreased to 0.24 kDa (DCs4), the Au colloids showed the longitudinal plasmon resonance.
absorption peak corresponding the long axis direction at 678 nm. The intensity at 678 nm was stronger than that at 526 nm, indicated AuNPs might aggregate and became larger non-spherical particles[8]. Fig 4 (B).~(E). are the TEM images of AuNPs prepared with 0.01%(w/v) DCs1, DCs2, DCs3 and DCs4, respectively. AuNPs prepared by DCs1, DCs2 and DCs3 were mainly spherical, the average particle size was 11.03±3.37 nm, 10.01±2.49 nm and 10.04±2.34 nm respectively, which indicated that the decrease of CTS molecular weight cannot obvious effect the size and morphology of AuNPs. AuNPs appeared by DCs4 showed serious gathering. Neutral protease hydrolyzes chitosan made it form more reduction activity sites, led to a lot of Au(III) was reduced to Au. However, CTS was not enough to stabilize large amounts of gold nuclear, and eventually caused aggregation growth of AuNPs[9].

Figure 4. (A)UV-Vis spectra of AuNPs prepared by 1.0 mmol/L HAuCl₄ solution with 0.01%(w/v) CTS with different molecular weight at 70°C for 2 h (Inset image: color of Au colloids prepared by CTS, DCs1, DCs2, DCs3 and DCs4 from left to right). TEM images of AuNPs synthesized using,(B)DCs1, (C)DCs2, (D)DCs3, (E)DCs4.

Conclusions

As for the one-step green preparation of AuNPs with chitosan, the spherical AuNPs with good dispersion, uniform particle size and single morphology were prepared under the experimental conditions of 70°C, 3 mL of 0.01%(w/v) chitosan with molecular weight in the range of 48.75 to 3.99 kDa, and 1 mL 1.0 mmol/L HAuCl₄. Besides, the change of molecular weight of CTS had no significant effect on the size and morphology of AuNPs. The substitution degree of CTS molecular weight affected the reduction ability of CTS to Au³⁺.

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References


